

Plant Gene Register

Nucleotide Sequence of a *Eucalyptus botryoides* Gene Encoding Cinnamyl Alcohol Dehydrogenase

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CAD (EC 1.1.1.195) catalyzes the reduction of hydroxycinnamic aldehydes, para-coumaryl, coniferyl, and sinapyl aldehydes to the corresponding alcohols (monolignols) (Higuchi, 1990). CAD has been purified from several plants (Lüderitz and Grisebach, 1981; Sarni et al., 1984; Halpin et al., 1992; O'Malley et al., 1992) and extensively characterized. Knight et al. (1992) isolated two cDNA clones encoding CAD from tobacco. We purified and characterized CAD from a perennial dicot *Aralia cordata* (Hibino et al., 1993b). An oligonucleotide derived from a partial amino acid sequence was used as a probe to isolate a cDNA encoding CAD of *A. cordata* (Hibino et al., 1993a). The amino acid sequence derived from the cDNA shares homology with those derived from the cDNAs encoding tobacco CAD.

The cDNA isolated from *A. cordata* was used as a probe to isolate a eucalyptus CAD gene, designated ECAD21, from a genomic library derived from shoot primordia of *Eucalyptus botryoides*. The *E. botryoides* CAD gene contains four introns and five exons that encode a polypeptide of 355 amino acids (Table I). The mol wt is calculated to be 38,765. The region upstream of the initiation codon contains a putative TATA box sequence, three CAT box sequences (CAAT), and two direct repeat sequences.

Goffner et al. (1992) also isolated and characterized two CAD proteins from *Eucalyptus gunii* (CAD1 and CAD2). Recently, they reported isolation of a cDNA clone, designated ECAD2, encoding the *E. gunii* CAD (CAD2) (Grima-Pettenati et al., 1993). The amino acid sequence derived from the *E. botryoides* CAD gene is 97% homologous with that derived from the *E. gunii* CAD2 cDNA.

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Abbreviation: CAD, cinnamyl alcohol dehydrogenase.

Table I. Characteristics of the *Eucalyptus* CAD gene

Organism:
<i>Eucalyptus botryoides</i> .
Gene Product, Pathway:
A subunit of CAD (EC 1.1.1.195); lignin biosynthesis.
Techniques:
A genomic clone was isolated from a genomic library derived from <i>E. botryoides</i> using a cDNA encoding CAD from <i>A. cordata</i> as a probe. A 3.3-kb <i>Sal</i> I fragment was cloned into pUC19 for sequence analysis. The dideoxy chain termination method was used to completely sequence both strands.
Gene Identification:
Sequence comparison of the deduced amino acid sequence with that deduced from an <i>A. cordata</i> CAD cDNA (Hibino et al., 1993a).
Features of Gene Structure:
Five exons and four introns are contained in amino acid-coding region.
Structural Features of Protein:
Protein contains 355 amino acid residues; <i>M</i> _r 38,765. A consensus sequence of zinc-binding domain is found from Gly ⁶⁸ to Val ⁸²

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